Page 2
DETAILED ACTION
Election/Restrictions

Applicant's election without traverse of Group IV in Paper No. 6 is acknowledged.
Claim Rejections - 35 USC § 112
2. Claims 10 and 20-27are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is vague and indefinite what is meant by the phrase "one of which lyses" in claim 10. Specifically, "lyses" what?. The precipitation reagent must precipitate DNA, but there is no similar limitation placed on the lyses reagent.
The phrase "the method" in claim 20 lacks antecedent basis.

Claim 20 [is] rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential elements, such omission amounting to a gap between the elements. See MPEP § 2172.01. The omitted elements are: what comprises the apparatus for application of the method in parallel.

It is vague and indefinite what are the metes and bounds of a "stripping solution" of a "resuspension solution" or of a "lysis solution" as listed in claim 21 and later claims. Specifically, no structural components are indicated for these solutions so potentially any solution can meet these phrases.

Claim Rejections - 35 USC § 102
3. The following is a quotation of the appropriate paragraphs of 35 U.S.C.102 that form the basis for the rejections under this section made in this Office action:
Application/Control Number: 09/841,763 Art Unit: 1637
Page 3
A person shall be entitled to a patent unless -
(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United

4. Claims 10 is rejected under 35 U.S.C. 102(b) as being anticipated by Morris et al (J. Gen. Microbiol. (1978) 106:387-389).
As an initial matter prior to the rejection, the claims must be construed. These claims are construed using the broadest reasonable interpretation where the intended use limitations are not given patentable weight since they do not limit the compositions in any structural sense.
Morris teaches a lysis composition which comprises 5 mM spermidine, to which is added the detergent SDS which is a lysing reagent (see page 387, paragraph 2).
Claim Rejections - 35 USC § 103

The present invention precipitates a DNA product contaminated with less than 10% RNA, preferably in a single step, and without use of proteinase or nucleases. The selective precipitation of the invention directly harvests nucleic acids and the target nucleic acid of a precipitation can be changed by changing conditions (i.e. type of compaction agent, quantity of compaction agent, concentration of salts, etc.) Because of this selectivity other large biomolecular contaminants such as proteins, unwanted nucleic acids,

carbohydrates, etc. do not have to be degraded by enzymes. Thus the use of troublesome RNAse, DNAse, proteases, and other enzymes (with their attendent difficulty in cleanup and prevention of cross-contamination) is avoided by the invention.

Morris' "cold lysis buffer" contains spermidine, but not to precipitate anything. Instead Morris uses a many-stepped procedure which does require the troublesome enzymes. Morris adds SDS in a second step *after* the lysis ("rupture") of his cells is complete (See Morris p. 137, 2nd paragraph particularly lines 3-4 and 15.). Thus Morris teaches no "composition comprising a mixture of combined reagents one of which lyses and another of which precipitates..." according to original Claim 10. In fact, by employing two steps using two different reagents, Morris "teaches away" and makes it even less obvious that a single combined reagent could accomplish the advantages of the present invention. The Morris reference "would likely discourage the art worker from attempting ..." the mixture of reagents taught by the inventors and claimed in Claim 10. (See Gillette Co. v. S.C. Johnson, 16 USPQ2nd 1923 (Fed. Cir. 1990).

According to "Mama Ji's Molecular Kitchen", accessed by Internet Explorer via Google search for "SDS detergents":

> "SDS stands for sodium dodecyl (lauryl) sulfate. The purpose of the SDS detergent is to take the protein from its native shape, which is basically a big glob, and open it up into a linear piece. It's kind of like taking a wadded up ball of string and untangling it into one straight, long piece. This will allow it to run more efficiently down the gel and will get you better results, since it's easier to compare two linear pieces of something rather than two wads of the same thing.
>
> "In more scientific terms, it is an anionic detergent that binds quantitatively to proteins, giving them linearity and uniform charge, so that they can be separated solely on the basis if

> their size. The SDS has a high negative charge that overwhelms any charge the protein may have, imparting all proteins with a relatively equal negative charge. The SDS has a hydrophobic tail that interacts strongly with protein (polypeptide) chains. The number of SDS molecules that bind to a protein is proportional to the number of amino acids that make up the protein. Each SDS molecule contributes two negative charges, overwhelming any charge the protein may have. SDS also disrupts the forces that contribute to protein folding (tertiary structure), ensuring that the protein is not only uniformly negatively charged, but linear as well.

Contrast the above with the following regarding lysis, from the same source:

> Alkaline lysis is the method of choice for isolating circular plasmid DNA, or even RNA, from bacterial cells. It is probably one of the most generally useful techniques as is a fast, reliable and relatively clean way to obtain DNA from cells. If necessary, DNA from an alkaline lysis prep can be further purified.
>
> Alkaline lysis depends on a unique property of plasmid DNA. It is able to rapidly anneal following denaturation. This is what allows the plasmid DNA to be separated from the bacterial chromosome.
>
> Typically, you will grow up E coli cells that contain the plasmid you want to isolate, then you will lyse the cells with alkali and extract the plasmid DNA. The cell debris is precipitated using SDS and potassium acetate. This is spun down, and the pellet is removed. Isopropanol is then used to precipitate the DNA from the supernatant, the supernatant is removed, and the DNA is resuspended in buffer (often TE). A mini prep usually yields 5-10 ug. This can be scaled up to a midi prep or a maxi prep, which will yield

much larger amounts of DNA (or RNA).

Specific protocols for alkaline lysis differ widely from lab to lab, and even from scientist to scientist. The basic principles behind the procedure, however, are fairly uniform. Here they are:

1. Spin down your cells. Your DNA is still in the cells, so it is in the pellet at this stage.

2. Discard the supernatant. Pieces of cell wall are released from the bacteria and are floating around in the supernatant. These cell wall pieces can inhibit enzyme action on your final DNA, so it is important to get rid of all of the supernatant and to even invert the tube and wipe the lip with a Kim-wipe or Q-tip.

3. Resuspend the cells in buffer (often Tris) and EDTA. EDTA chelates divalent metals (primarily magnesium and calcium). Removal of these cations destabilizes the cell membrane. It also inhibits DNases. Glucose should also be added to maintain osmolarity and prevent the buffer from bursting the cells.

4. Lyse the cells with sodium hydroxide (NaOH) and SDS. This highly alkaline solution gave rise to the name of this technique. Mix this by gentle inversion and incubate on ice for five minutes (but no longer, or your DNA will be irreversibly denatured). Three things

happen during this stage:

a. SDS pops holes in the cell membranes. SDS (sodium dodecyl (lauryl) sulfate) is a detergent found in many common items such as soap, shampoo and toothpaste.

b. NaOH loosens the cell walls and releases the plasmid DNA and sheared cellular DNA.

c. NaOH denatures the DNA. Cellular DNA becomes linearized and the strands are separated. Plasmid DNA is circular and remains topologically constrained.

5. Renature the plasmid DNA and get rid of the garbage. Add potassium acetate (KAc), which does three things:

a. Circular DNA is allowed to renature. Sheared cellular DNA remains denatured as single stranded DNA (ssDNA).

b. The ssDNA is precipitated, since large ssDNA molecules are insoluble in high salt.

c. Adding sodium acetate to the SDS forms KDS, which is insoluble. This will allow for the easy removal of the SDS from your plasmid DNA.

Now that you've made it easy to separate many of the contaminants, centrifuge to remove cell debris, KDS and cellular ssDNA. Your plasmid DNA is in the supernatant, while all of the garbage is in the pellet.

6. Precipitate the plasmid

DNA by alcohol precipitation (ethanol or isopropanol) and a salt (such as ammonium acetate, lithium chloride, sodium chloride or sodium acetate) and spin this down. DNA is negatively charged, so adding a salt masks the charges and allows DNA to precipitate. This will place your DNA in the pellet.
7. Rinse the pellet—your plasmid DNA—in ice-cold 70% EtOH and air-dry for about 10 minutes to allow the EtOH to evaporate.
8. Resuspend your now clean DNA pellet in buffer (often Tris) and EDTA plus RNases to cleave any remaining RNA. Your DNA is now back in solution.
DNA of this purity is good for a number of uses, such as in vitro transcription or translation or cutting with some enzymes. If you are sequencing or transforming this DNA into mammalian cells, you'll want to use additional purification techniques such as phenol extraction, Qiagen column purification, or silica-based purification." (Emphasis added.)

Note that Morris' alkaline lysis with SDS requires further purification to achieve the purity of the products of the present invention.

In fact Morris (p. 387 6th line above bottom) states "...and is then incubated with proteinase K...for 16 hours (overnight) at 42°C.", thus Morris is using using the very reagent avoided by the kit of the present invention. Enzymes are preferably avoided as reagents because of their attendant possibility for contamination and requirement of extensive cleanup.

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 10, 19-37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Morris et al (J. Gen. Microbiol. (1978) 106:387-389) in view of Stratagene Catalog (1988) p. 39.
Morris teaches a lysis composition which comprises 5 mM spermidine, to which is added the nonionic detergent SDS which is a lysing reagent (see page 387, paragraph 2).

Morris teaches many separate different reagents which are added to his culture at different times. One of Morris' reagents includes spermidine and another of Morris' reagents is SDS. (See Morris p. 137, 2nd paragraph particularly lines 3-4 and 15.)

Morris further teaches the use of glass centrifuge tubes, which is an apparatus which permits application of the method in parallel as well as the use of centrifugation apparatus (see page 387, paragraph 2). In a kit, as taught by Stratagene below, multiple vials of the same solution are commonly used to minimize contamination.

Morris' mention of "a 30 ml siliconized glass centrifuge tube" in 1977 (Morris p. 387, 2nd para., line 2) does not suggest application of his method in parallel and there is no such suggestion elsewhere in Morris.

Morris teaches a first compaction precipitation solution which is the cold lysis buffer with spermidine (see page 387)
Page 4
a stripping solution, which is the phenol solution (see page 387) a Tris resuspension solution (see page 387) and a second compaction solution, which is the PEG solution (page 388)

In his first two steps, Morris teaches two separate different reagents which are added to his culture at different times. One of Morris' reagents includes spermidine and another of Morris' reagents is SDS. (See Morris p. 137, 2nd paragraph particularly lines 3-4 and 15.) Later, Morris' lysate is incubated with proteinase and then "deproteinized" with phenol/Tris HCl and then treated by "dialysis". (p. 387 lines 6 above bottom to bottom.).

Morris teaches filtering the nucleic acid through an agarose gel (see figure 1 on page 388) as well as the apparatus for its use (see figure 1 on page 388).

It is respectfully urged that the electrophoresis shown in Fig. 1 of Morris would not be considered "filtering" by a person skilled in the art.

Morris only uses spermidine to stabilize DNA during his shear-induced spore rupture procedure (using glass beads and a vortex), keeping the cellular DNA in a compact nuclear morphology until lysis in detergent. Morris' spores are then harvested, not by precipitation, but by adding 3-4 mLs of the spermidine-containing lysis solution and his spermidine is not stripped by this solution since he is adding *additional* spermidine. Then Morris adds 1% SDS, then his solution is heated to 60°C for 20 minutes (continuing lysis) and incubated with Protease K (an enzyme used to break down proteins) overnight at 42°C (This also could assist in lysis). Then Morris performs a phenol extraction (2-phase organic extraction) to remove protein into his hydrophobic organic (phenol) phase. At this point Morris removes residual phenol with a dialysis and the dialysis buffer containing RNAse. Morris' dialysis is carried out at 42°C for 1 hour to allow the RNAse to digest any residual RNA. Then his batch is dialyzed against PEG to concentrate the batch. This is a common method for concentration of solutions since the PEG concentrations attempt to equalize so water permeates out of the dialysis bag, in effect concentrating the solution in the dialysis tubing. (PEG is not used to precipitate DNA in this Morris reference). Then a final dialysis is done by Morris to change buffers and remove any small molecules remaining from his two enzymatic digestion steps.

To sum up, Morris' spermidine is not used in any way for a purification. In addition, PEG is used only to concentrate Morris' batch via dialysis, not to purify the cellular DNA. Morris' removal of proteins is done with protease K and a phenol extraction while Morris' RNA is removed by using RNAse.

Morris uses the very enzymes that the invention avoids!

> *Morris does not teach placement of these reagents into a kit format.*
> *Stratagene catalog teaches a motivation to combine reagents into kit format (page mu),*
> *It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Morris into a kit format as discussed by Stratagene catalog since the Stratagene catalog teaches a motivation for combining reagents of use in an assay into a kit, "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantitites of 10 different reagents, each of which is needed in only microgram amounts, when beginning*
> *Page 5*
> *a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantitites you will actually need, premixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control" (page 39, column 1).*

The Stratagene reference is just a product catalog and merely shows that kits are already available for some other biotech procedures. The combination of Morris and Stratagene still would not show the simplicity of the reagents of the present invention, much less the combination of mixed or simultaneously added reagents as claimed in present Claim 10. Further, because Morris requires about seven different reagents plus three dialysis steps, combining Stratagene with Morris would make a cumbersome kit; absent improper hindsight gained by a reading of the present application. The discovery that the present invention can avoid all of this is surely unobvious according to 35 USC 103. Claim 21 now recites "consisting essentially of" to emphasize the simplicity of the invention.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jeffrey Fredman whose telephone number is 703-3086568. The examiner can normally be reached on 6:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 703-308-1119. The fax phone numbers for the organization where this application or proceeding is assigned are 703-305-3014 for regular communications and 703-305-3014 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-3080196.

Jeffrey Fredman
Primary Examiner
Art Unit 1637
August 1, 2002

The claims have been clarified and broadened merely by addition of wording from the original specification; no new matter has been added and no estoppel is involved. The changes were not required by the art cited because the original claims themselves distinguished from the references relied on. No references were cited which were not relied on for rejections.

The fee [$460.00] for the three month extension fee and any necessary (small entity) charges can be charged to USPTO Deposit Account 20-336 of Technology Licensing Co. LLC. Correspondence may be addressed to Customer No. 26830.

The Examiner is especially invited to telephone Applicants' Attorney if that would expedite prosecution and disposal of this Application.

Respectfully submitted,

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